
Article:
Ciano, Luisa orcid.org/0000-0002-1667-0856, Davies, Gideon John orcid.org/0000-0002-7343-776X, Tolman, William et al. (1 more author) (2018) Bracing copper for the catalytic oxidation of C-H bonds. Nature Catalysis. pp. 571-577.

https://doi.org/10.1038/s41929-018-0110-9

Bracing copper for the catalytic oxidation of C–H bonds

Luisa Ciano,[a] Gideon J Davies,[a] William B Tolman,[b,*] Paul H Walton[a,*]

[a] Department of Chemistry, University of York, Heslington, York, UK, YO10 5DD.

[b] Department of Chemistry, One Brookings Hall, Campus Box 1134, Washington University in St. Louis, St. Louis MO 63130-4899, USA.

E-mail: wbtolman@wustl.edu, paul.walton@york.ac.uk

Abstract

A structural unit found in the active site of some copper proteins, the histidine brace is comprised of an N-terminal histidine which chelates a single copper ion through its amino terminus NH_2 and the π-N of its imidazole side chain, and coordination by the τ-N of a further histidine side chain, to give an overall N_3 T-shaped coordination at the copper. The histidine brace appears in several proteins, including *lytic polysaccharide monooxygenases* (L)PMOs and *particulate methane monooxygenases* pMMOs, both of which catalyse the oxidation of substrates with strong C–H bonds (bond dissociation enthalpies ~ 100 kcal/mol). As such, the copper histidine brace is the focus of research aimed at understanding how Nature catalyses the oxidation of unactivated C–H bonds. In this Perspective, we evaluate these studies, which further give bioinspired direction to coordination chemists in the design and preparation of small molecule copper oxidation catalysts.

Introduction

Discovered in 2011, the histidine brace[1], or His-brace ("brace" from the old English noun meaning "a pair" and the verb "to clasp") is the name given to a structural unit found in the active site of copper enzymes. It is comprised of an N-terminal histidine which chelates to a single copper ion through its NH_2 group and the π-N (also defined as δ-N) of its imidazole side chain. Coordination is completed by the τ-N (also, ε-N) of a further histidine side chain to give an overall T-shaped N_3 coordination geometry at the copper ion (Figure 1). The histidine brace appears in the proteins *lytic polysaccharide monooxygenases* (LPMOs; also termed PMOs[2]), the copper transport protein *CopC*,[3] and *particulate methane monooxygenases* (pMMOs). Both LPMOs and pMMOs have roles in the terrestrial biological carbon cycle and have major industrial uses/potential. LPMOs catalyse the O_2 and co-factor-dependent oxidation of polysaccharides, leading to cleavage of a glycosidic bond,[1, 4] and attract attention because of their utility in commercial biofuel production.[5, 6, 7, 8] pMMOs catalyse the oxidation of CH_4 by O_2,[9] and are enzymes of importance because of their potential to convert methane into a liquid fuel (e.g. MeOH).

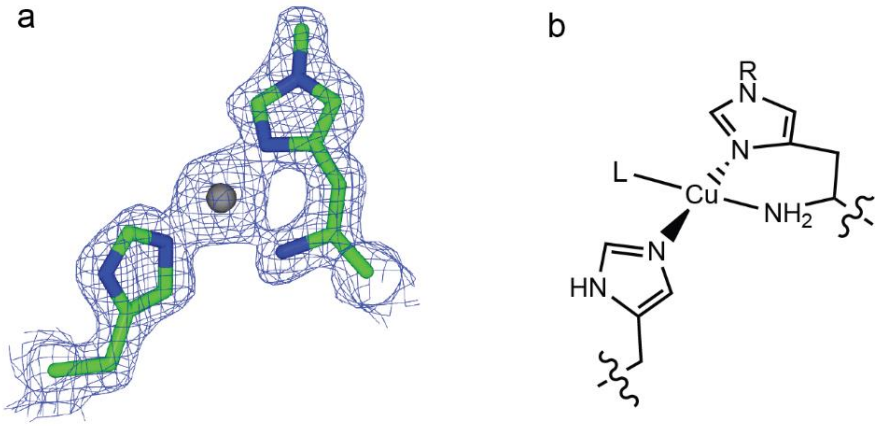

Figure 1. The copper histidine brace. a) Electron density contour map of histidine brace active site from an exemplar Cu(I)-AA9 LPMO (PDB code 5ACH), b) the copper histidine brace (R = Me or H).

Beyond their biological and industrial importance, both pMMO and LPMOs are of interest at a molecular level since they catalyse the oxidation of a strong (bond dissociation enthalpy ~95-103 kcal/mol) C–H bond,[7, 10] at which the carbon atom is saturated. Thus, any oxidative mechanism employed by the enzymes requires the generation of highly oxidising intermediates. Accordingly, central questions surround the histidine brace, including how it facilitates the generation of such highly oxidising intermediates and what are their structural and electronic features. We address these and related questions in this Perspective by interweaving discussions of enzymatic structure/function relationships with those of synthetic model complexes. Our objective is to highlight the tremendous catalytic potential of enzymes or molecules that contain the histidine brace and the importance of this ligating unit on their possible mechanisms of action.[11]

Discovery of the copper histidine brace

The first reports[12, 13] of a new type of "non-catalytic" protein that bound to polysaccharides appeared in 2005. It was evident from crystal structures of these proteins that they had a metal-binding site in which a conserved N-terminal histidine chelated a metal ion. Later studies then demonstrated that these proteins were enzymes (LPMOs) that catalysed the oxidative degradation of polysaccharides and that the metal was essential for any activity.[14] During this time the metal ion was variously assigned as Ca, Na, Zn and Mg,[4, 13, 14, 15] incommensurate with the oxidoreductase properties of the enzyme. Adding to the confusion and highlighting the need for a small molecule understanding of metal-containing active sites, other studies had also proposed that the redox-active transition metals Co, Ni and Mn could also bind at the active site.[14] The true identity of the metal as copper was determined in 2011 through the use of careful metal-ion binding studies, EPR spectroscopy[1] and activity studies.[2] This work[1] was the also first to name the active site as the histidine brace, a moniker now in wide use. These studies[1] further revealed that the copper histidine brace in LPMOs isolated from fungal sources features an N-methylation on the N-terminal histidine, a feature which had also been missed in earlier crystallographic studies.[14, 15] LPMOs are now known to be widespread in Nature,[16] with over 3500 examples already identified in the CAZy database for carbohydrate-active proteins.[17] The enzymes have been classified on the basis of their amino-acid sequences into six distinct classes and given the codes AA9, AA10, AA11,[18] AA13,[19, 20] AA14[21] and AA15[22] (Figure 2).

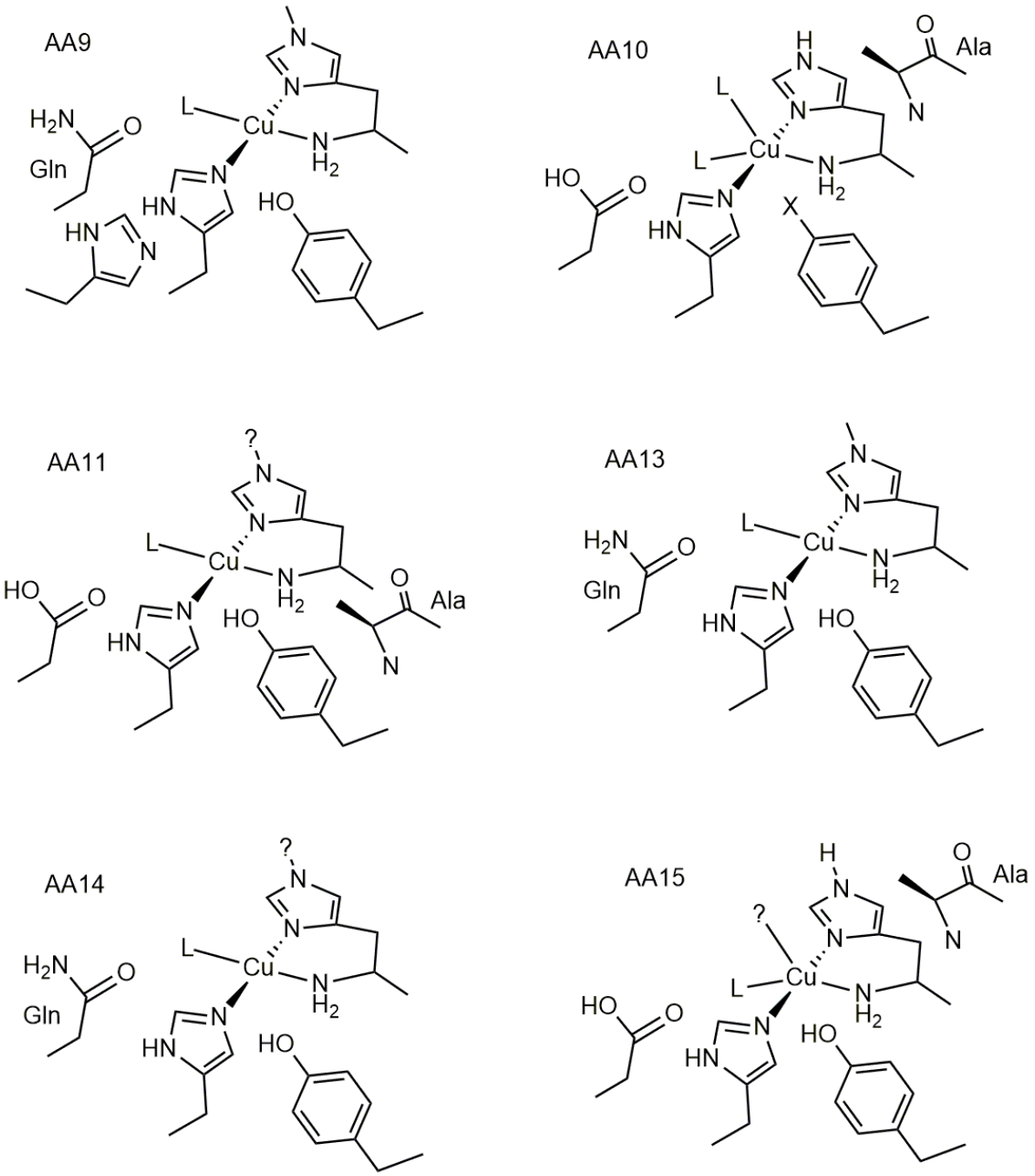

Figure 2. LPMO active sites. Cu(II) histidine brace structures from the active sites of LPMOs (X = H or OH in AA10s, L = H_2O or OH^-), depicting some of the conserved secondary coordination sphere amino acid side chains. The wild type AA11 and AA14 LPMOs may contain a histidine side chain which is methylated, but this is unknown since the expression system (*Pichia pastoris*) used to express these genes appears to lack the necessary methylation apparatus.

The structure of the copper histidine brace in LPMOs

Various reviews have compiled the biochemical and catalytic aspects of LPMOs.[6, 16, 23] These commentaries highlight the structural range of the active sites found within the enzymes, as shown not only from the 45 known crystal structures, but also the various EPR spectroscopy studies.[23, 24] This diversity is evident in the identity and relative positions of amino acids in the secondary coordination spheres of the copper ion which vary to a significant degree from one LPMO class to

another.[25] The only common structural aspect of these LPMOs is the T-shaped coordination geometry of the histidine brace—a feature recognised in recent work on artificial copper-metalloenzymes.[26] In fact, there is so little structural variation in the primary coordination sphere of the copper (Supplementary Table 1) that the copper histidine brace may be viewed as a geometrically-fixed structural unit, the reactivity of which is finessed through secondary coordination sphere interactions.[24, 27] Of these secondary interactions, those amino acid side chains which are capable of forming hydrogen bonds to any exogenous substrate bound to the copper ion are of particular importance insofar as highly reactive oxygen-based ligands (e.g superoxide, hydroperoxide, oxyl) are potentially affected through such interactions. Indeed, site-directed mutagenesis studies have already implicated the importance of the secondary glutamine and histidine residues in AA9 LPMOs.[25] Other residues, for example the tyrosine in AA9 LPMOs (Figure 2), also have significant effects on catalytic rates/turnover numbers, while others near the active site (e.g. alanine in AA10 LPMOs) may have roles in controlling access of exogenous ligands to the axial coordination site on the copper,[14, 27] although these residues do not appear to affect the regioselectivity of polysaccharide oxidation.[28] In terms of secondary interactions, a recent crystal structure of an AA9 LPMO in contact with its saccharide substrate[29] revealed the details of substrate-active site interactions which modulate the electronics and therefore reactivity of the Cu histidine brace.[6] Included in these changes are an increased covalency in the metal-ligand interactions and a change in hydrogen bonding between the amino terminus and a nearby water molecule. In this context,[30] an on-going debate within the field is whether substrate binding is coupled to the activity of the enzyme, such that the generation of highly oxidising copper species that carry out site-specific oxidation on the substrate only takes place in the presence of substrate (as is the case of cytochrome P450, for example).[31]

The primary coordination geometry around the copper in the histidine brace might be as expected from a straightforward consideration of the lone pair orbitals of the coordinating nitrogen atoms. Two *trans* sp^2-hybridised nitrogen atoms coordinate with average distances of Cu(II)–N(1) and Cu(II)–N(2) ~2.0 Å (Supplementary Tables 1 and 2). The angle of twist between the two best fit planes associated with each of the imidazole rings of the two histidines is around 65° and appears to be dependent on the presence of the metal ion, as the angle is around 30° in most of the *apo* structures available (AA10s). This twist angle is significantly different from the analogous angle found in many *trans*-N-heterocycle Cu(II) complexes, where it is usually less than 30°, a result of the conformational demands of the chelate rings of ligands (Supplementary Table 3). The significance of this difference is unclear. If, however, such a twist angle influences the reactivity of the copper histidine brace, particularly in copper-oxygen intermediates, then some role for the π-interaction capacities of the N-heterocycles seems to be worth consideration in the design of ligands aimed at emulating the active site. The coordination sphere of the copper in the histidine brace is completed from coordination in the 'bridgehead' position by the NH_2 amino terminus of the protein. In accord with the sp^3 nature of the lone pair on this group, the average Cu–N(amino) bond length from X-ray structures is ~2.2 Å, although some care must be exercised in reading too much into this distance since the structures of LPMOs are bedevilled by photo-reduction of the Cu(II) ion to Cu(I) during X-ray diffraction experiments.[27, 32] For instance, the published DFT calculations of the histidine brace consistently calculate the Cu–NH_2 distance to be 2.1 A, some 0.1 A shorter than those usually measured in crystal structures.[33]

The histidine brace N_3 coordination is such that the N–Cu–N angles all fall within the range 87° to 106°, averaging around 94°. From this feature it is reasonable to assume that the lone pairs of the nitrogen atoms are maximally overlapped with the d(x^2–y^2) orbital of the copper, necessarily requiring that the redox active molecular orbital (RAMO) on the copper is at high energy compared

to the rest of the d-orbital manifold. Such a high energy redox-active orbital has implications for the reactivity of the copper ion, the first of which is that the strength of the copper(II)-superoxide bond [formed upon initial reaction of O_2 with Cu(I)] is maximised through a lowered difference in energy between the frontier orbital on the copper d(x^2–y^2) and the oxygen (anti-bonding π_g). The high strength of the bond offsets the energetic penalty associated with the one-electron reduction of O_2 by Cu(I).[34] A second feature of the d-orbital configuration of the histidine brace is that any d–orbital mixing can be expected to be low, lending the RAMO near pure d(x^2–y^2) orbital character, as supported by the values of hyperfine coupling constants observed in the EPR spectra of LPMOs in the presence of substrates.[29] We therefore speculate that any inner-sphere redox chemistry on the exogenous ligands associated with the copper is most likely to occur with ligands in the equatorial plane of the copper coordination sphere, thus hindering what might be potentially deleterious generation of reactive oxidation species through electron transfer mechanisms at the axial position of the copper ion.[35]

An ongoing debate about the mechanism of LPMOs has included discussion about the protonation state of the NH_2 binding unit. There is some circumstantial evidence from studies of synthetic complexes that deprotonation of this group could occur. For instance, Cu(III) oligopeptide species exhibit pK_as for Cu(III) –NH_2 amino terminus moieties of ca. 8.8, a value approaching biological viability.[36] Also, $[CuOH]^{2+}$ complexes stabilised by anionic amidato type ligands are competent for hydrogen atom abstraction from C-H bonds with strengths of >90 kcal mol^{-1} (see below). Accordingly, deprotonation of the NH_2 in LPMOs to give an imido group gains credence, and such a possibility was raised in the 2011 paper which announced the copper histidine brace active site in LPMOs.[1] The notion gained further momentum from neutron diffraction experiments performed on AA10 LPMOs, which appeared to show a lack of density around the amino terminus nitrogen atom[37] and a notable average Cu–NH_2–Cα angle of ~117°; confirmation by spectroscopy is nonetheless needed before a definitive assignment can be made. If true, however, the enhanced electron donation by the deprotonated moiety would raise the d(x^2–y^2) orbital high enough in energy such that a formal Cu(III) species becomes realistic.[36] On the other hand, recent DFT calculations on an LPMO in the presence of its oligosaccharide substrate suggested that deprotonation of the amino terminus in LPMOs potentially reduces the capacity of a $[CuOH]^{2+}$ species to perform hydrogen atom abstraction.[33] If a deprotonated amino group is an essential feature of the reactivity of the copper histidine brace, then its presence presents challenges for ligand design for small molecule mimics, since the NH_2 'bridgehead' in ligand design is commonly used as an anchor point for the nitrogen heterocycles (usually linked to the nitrogen atom of the bridgehead through a methylene or ethylene linker group).

The structure of the copper histidine brace in pMMO

The exact nature of the active site species responsible for attacking the strong C–H bond in methane in pMMO is controversial.[38, 39, 40] Early EXAFS, XRD and EPR work on this enzyme had concluded that the active site contains not a single copper ion, but two in close proximity to each other (Cu…Cu, ca 2.6 Å).[38, 41, 42, 43, 44] An alternative proposal suggested that the active site was a tricopper cluster.[40] These hypotheses, supported by DFT investigations,[45, 46, 47] spurred many scientists to investigate multicopper structures and their analogous multicopper-oxygen complexes as potential model complexes, catalytic or not, of the active site of pMMO. [39, 48, 49 9, 38, 43, 50] However, notwithstanding the extensive work into multi-copper centers and in the light of the discovery of the histidine brace, there is now growing evidence in support of an alternative proposal in which pMMO contains a mononuclear Cu active site. Indeed, the 2011 discovery of the histidine brace in LPMOs prompted a specific proposal from the authors that the active site of pMMO could be mononuclear in copper

and that such a possibility 'warranted investigation'.[1, 5] Further work on LPMOs by some of the same authors showed that the EXAFS spectrum of Cu(II)-LPMO was similar to that reported for pMMO, lending further weight to the possibility of a mononuclear copper active site in pMMOs.[34] Since then, other commentators have drawn attention to the unusual, if not unreasonable, coordination geometry of the active site histidines of pMMOs if two copper ions are to be accommodated therein, as illustrated in the original X-ray structure by the angle made by the 'coordinating' histidine side chains to the two coppers (Figure 3).[39] Most revealingly, and perhaps stimulated by the discovery of LPMOs, a reappraisal of the original X-ray data of pMMO (3RGB) by some of the original presenters of the dicopper model using quantum crystallographic methods, led to the conclusion that the electron density at the active does not support a two copper assignment, and that the data are best interpreted as a mononuclear copper active site.[51] This study was coupled to a DFT calculation of methane oxidation by a mononuclear copper site (a $[CuO]^+$ species, as discussed below) coordinated by the histidine brace, also arriving at the conclusion that oxidation of methane could occur without significant activation barriers. Furthermore, a very recent EXAFS and EPR study has backed-up the findings from the quantum crystallographic work.[52] With the weight of evidence now swinging away from a dinuclear active site within pMMO and with the discovery of the histidine brace in LPMOs, there are major repercussions for our understanding of the mechanism of O_2 activation and substrate oxidation at monomeric copper sites. Moreover, given that the C–H bonds which both LPMOs and pMMOs oxidise are strong and unactivated, the postulate of a histidine brace coordinating a single copper is inspiring coordination chemists interested in preparing synthetic copper complexes which themselves could then be used as powerful oxidation catalysts.[53]

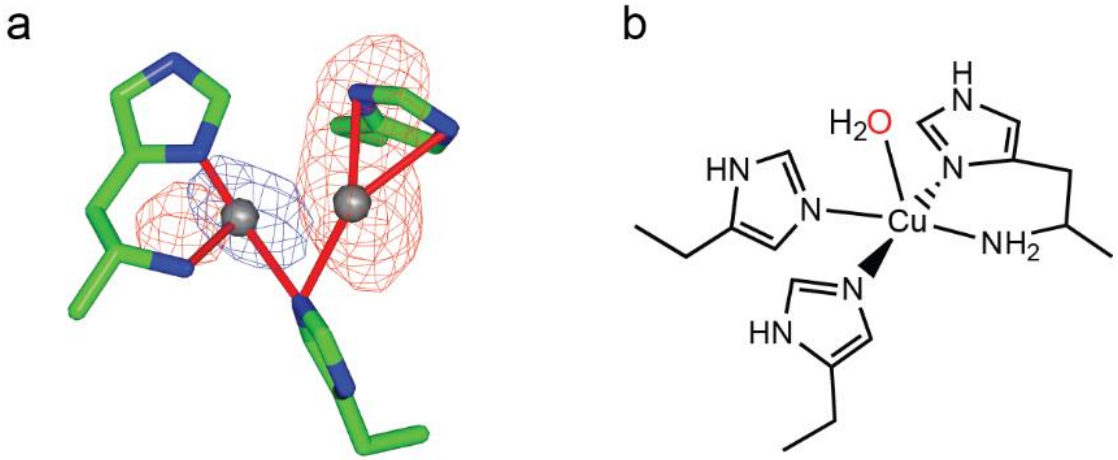

Figure 3. The changing structure of the active site of pMMO. a) Original electron density X-ray maps from one pMMO structure (3RGB),[50] showing non-canonical bonding arrangements of histidines (Cu-His bonds shown as red cylinders), a single $2F_o–F_c$ peak for a mononuclear Cu and large regions of negative $F_o–F_c$ electron density associated with the modelling of two Cu positions, b) proposed structure of the pMMO site as now re-evaluated by quantum crystallography.[51]

Mechanistic Considerations

Proposals exist for the generation of free hydroxyl radicals by LPMOs, but—while certainly viable from the perspective of oxidative cleavage of a recalcitrant substrate—these notions appear incommensurate with the observed site-specific attack.[54] Attention, therefore, has focussed on reactive copper-oxygen species as the key oxidative intermediate(s). There is currently no single consensus on the mechanism by which LPMOs and pMMOs activate O_2 (or H_2O_2)[54] to hydroxylate

strong C–H bonds. Contributing to the lack of agreement for LPMOs is the fact that different LPMOs classes may employ different oxidative mechanisms during catalytic turnover.[6, 23] There are also different opinions as to whether O_2 or H_2O_2 is the natural substrate for LPMOs.[54, 55] The absence of a consensus may also reflect the inherent difficulty of studying what are naturally highly reactive intermediates, further highlighting the importance of small molecule studies that are capable of providing precedent for structural proposals. For example, the reaction of H_2O_2 with mono-copper complexes is known from small molecule studies to be complicated by unintentional reaction pathways.[56] Such pathways confound what might otherwise be simple mechanistic interpretations of how oxidised products are generated from the reaction with H_2O_2[54]—a caution also made by other authors.[8, 56] In this regard, studies of synthetic copper-oxygen complexes provide fundamental structural, spectroscopic, and mechanistic information relevant to their viability as intermediates in LPMOs and pMMOs.[49, 57, 58, 59, 60] Yet, compared to the plethora of multicopper-oxygen complexes that have been characterised, relatively few mononuclear compounds have been isolated, in large part due to the propensity of such small molecule species to form complexes with oxo, hydroxo, or peroxo bridges. A key synthetic strategy thus has been to use sterically hindered supported ligands to inhibit di- or multicopper complex formation.[61, 62, 63, 64]

(a) Intermediates with intact O-O bond

Several commentators have proposed what the key oxidative species might be in LPMOs.[6, 39] A Cu(II)-superoxide ($[CuO_2]^+$) intermediate is proposed to form upon reaction of a Cu(I) center with O_2 and surely merits further investigation in enzyme studies.[34, 65] Extensive synthetic studies of the reactions of appropriate Cu(I) precursors with O_2 led to the identification of $[CuO_2]^+$ species that may adopt multiple binding modes (η^1 and η^2) and extents of electron transfer to the bound O_2, bracketed by the resonance structure extremes of a Cu(II)-superoxide and a Cu(III)-peroxide.[60, 62, 63, 64, 66] Studies of the reactivity of such complexes with C–H bonds generally have indicated poor capabilities to perform hydrogen atom transfers (HATs), with most examples where HAT was observed involving attack at C–H bonds significantly weaker than those of the substrates of LPMO and pMMO.[59] While there are exceptions, the reactivity observed to date for $[CuO_2]^+$ compounds with exogenous substrates, along with results from theory,[67, 68] suggest that this core may not be responsible for attacking substrate C–H bonds in the enzymes. For this and other reasons, attention has focused on species further activated by the addition of protons and electrons.

Addition of one proton and one electron to the $[CuO_2]^+$ unit give a feasible intermediate with the core $[CuOOH]^+$. This concept is informed by the numerous examples of synthetic $[CuOOR]^+$ complexes which have been reported,[49, 69] with most prepared by the reaction of ROOH and base with suitable Cu(I) or Cu(II) precursors and characterised via spectroscopy; a few X-ray structures are available.[69, 70, 71] In a more biomimetic route, a $[CuOOH]^+$ intermediate was proposed to be formed by a proton-coupled electron transfer (PCET) of a $[CuO_2]^+$ complex with a 1-electron reductant (Fc or Fc*) in the presence of protic acid (trifluoracetic acid).[72] The recent identification of a $[CuOOH]^+$ unit stabilised by hydrogen bonds in an engineered protein is also a notable advance in this arena.[26] Examples of $[CuOOR]^+$ complexes reacting with C–H bonds are numerous, with reports of hydroxylations of strong sp^3 C–H bonds being particularly relevant here.[73] Most mechanistic proposals invoke O–O bond homolysis to yield a $[CuO]^+$ ("Cu(II)-oxyl") unit that is responsible for attacking the C–H bond.[74] Direct evidence for this pathway is scant, however, and differentiating this pathway from one involving direct hydrogen atom abstraction by the $[CuOOR]^+$ unit presents experimental challenges (in one study, a case for the latter pathway has been made).[75] Other work has shown that Cu–O bond homolysis can yield an alkylperoxo radical that attacks substrate.[56] While more reactivity and mechanistic studies of $[CuOOR]^+$ complexes are needed, the synthetic work

accomplished so far supports their feasibility as intermediates along O_2 activation pathways, but it is unclear whether they are sufficiently potent reagents to be responsible for the enzymatic C–H bond cleavage events.

(b) Intermediates derived from O-O bond scission

A key reactive intermediate in LPMOs proposed primarily on the basis of DFT calculations (of varying merit, see reference[23] for detailed comment) is the Cu(II)-oxyl, $[CuO]^+$.[63, 67, 76] Beyond LPMOs, such a species has long been proposed to be involved in monomeric copper oxidation catalysts/enzymes.[49, 77] The proposal of $[CuO]^+$ as a reaction intermediate is particularly appealing in view of the results of computational work that support it being highly reactive with strong C–H bonds[78, 79, 80] and experimental gas phase studies of the $[CuO]^+$ ion focused on its ability to hydroxylate methane.[81] Yet, no examples of complexes with this unit have been identified conclusively in condensed phase. It thus represents a synthetic 'holy grail', the attainment of which would provide important precedent for the involvement of this unit in oxidation catalysis.

In terms of LPMO mechanisms, recent DFT calculations have raised the possibility that the $[CuO]^+$ species in LPMOs can be formed following O-O bond scission of hydrogen peroxide by the Cu(I) form of the enzyme leading to the formation of the hydroxyl radical and a Cu(II)–OH unit, "caged" together within a cavity formed between the active site and substrate. Within the cage the hydroxyl rebounds to react with the $[Cu–OH]^+$ giving a $[CuO]^+$ and a water molecule.[68] While formation of the $[CuO]^+$ or hydroxyl radical requires the scission of the O–O bond in oxygen (or peroxide), a step which would be expected to have a high kinetic barrier due to the reorganisation energy associated with 1-electron reductive cleavage of a O–O bond, the calculated barrier for O–O scission is only 5.8 kcal/mol.[11] This normally prohibitive barrier is ostensibly reduced by the presence of a secondary coordination sphere glutamine in LPMOs (see above), which forms hydrogen bonds to the peroxide before O–O bond cleavage, thereby orientating O–O σ^* orbital towards the redox active d($x^2–y^2$) orbital on the copper.

From a small molecule perspective, well-characterised monocopper-oxygen intermediates lacking an O–O bond are limited to a protonated version of $[CuO]^+$, formulated as $[CuOH]^{2+}$. Examples of complexes with the latter have been prepared by 1-electron oxidation of $[CuOH]^+$ precursors (Figure 4).[82, 83, 84, 85, 86] Importantly as described above, the $[CuOH]^{2+}$ complexes (described formally as Cu(III)-OH species) are capable of attacking strong C-H bonds, and thermodynamic and kinetic studies have shed important light on the basis for this high reactivity. Particularly notable in this regard is the formation of a strong O–H bond (BDE ~90 kcal/mol) in the $[Cu(OH_2)]^{2+}$ product of hydrogen atom abstraction from substrate. This thermodynamic driving force may be directly related to the strong electron-donating character of the supporting ligand, which while mitigating the oxidising power of the complex enhances the basicity of the hydroxide. In essence, the ligand stabilises the high oxidation state of the $[CuOH]^{2+}$ core (enabling it to be observed and characterised) while at the same time increasing the thermodynamic driving force for hydrogen atom abstraction. These findings, as well as relevant theory,[67, 84] suggest that (a) the $[CuOH]^{2+}$ unit could be considered to be a feasible oxidant in LPMOs and pMMOs,[76] and (b) the role of the anionic ligand in stabilising the complexes while enhancing their reactivity with C–H bonds may be similarly filled by a deprotonated histidine brace in the enzymes. On the other hand, arguing against the $[CuOH]^{2+}$ unit as the active oxidant in the enzymes is the likely inability of the initial product of hydrogen atom abstraction, the $[Cu(OH_2)]^{2+}$ core, to undergo 'rebound' to yield the final hydroxylated organic product.

Other possible roles of the deprotonated histidine brace may be to enhance the basicity of (a) a $[CuO]^+$ species, potentially enhancing its HAT reactivity, or (b) the initially formed 1:1 adduct $[CuO_2]^+$.

Protonation of this adduct would yield a novel $[CuOOH]^{2+}$ group. The viability of this unit was recently supported by the preparation of complexes with $[CuOOR]^{2+}$ cores (R = cumyl, t-Bu) via 1-electron oxidation of $[CuOOR]^{+}$ precursors at low temperatures, using the same supporting ligand shown in Figure 4a (left, X = H).[69] While most $[CuO_2]^{+}$ complexes behave as electrophiles,[62] $[CuO_2]^{+}$ core supported by the electron donating dicarboxamide ligand exhibits nucleophilic character, presumably derived from the overall −1 charge of the complex.[87, 88] Taken together, the synthetic work provides some support for the notion that the deprotonated form of the histidine brace may facilitate O_2 activation and stabilise high-valent intermediates. These ideas are illustrated in Figure 4b, which features structural postulates for the initial 1:1 Cu/O_2 adduct, the $[CuOOH]^{2+}$ core that might be formed upon protonation of the adduct, and $[CuO]^{+}$ and $[CuOH]^{2+}$ cores, all stabilised by the deprotonated histidine brace (details of the mechanism of O-O bond scission by protons and electrons are omitted, but represent important targets of research). It is tempting to suggest that similar species may be involved in methane hydroxylation by pMMOs.

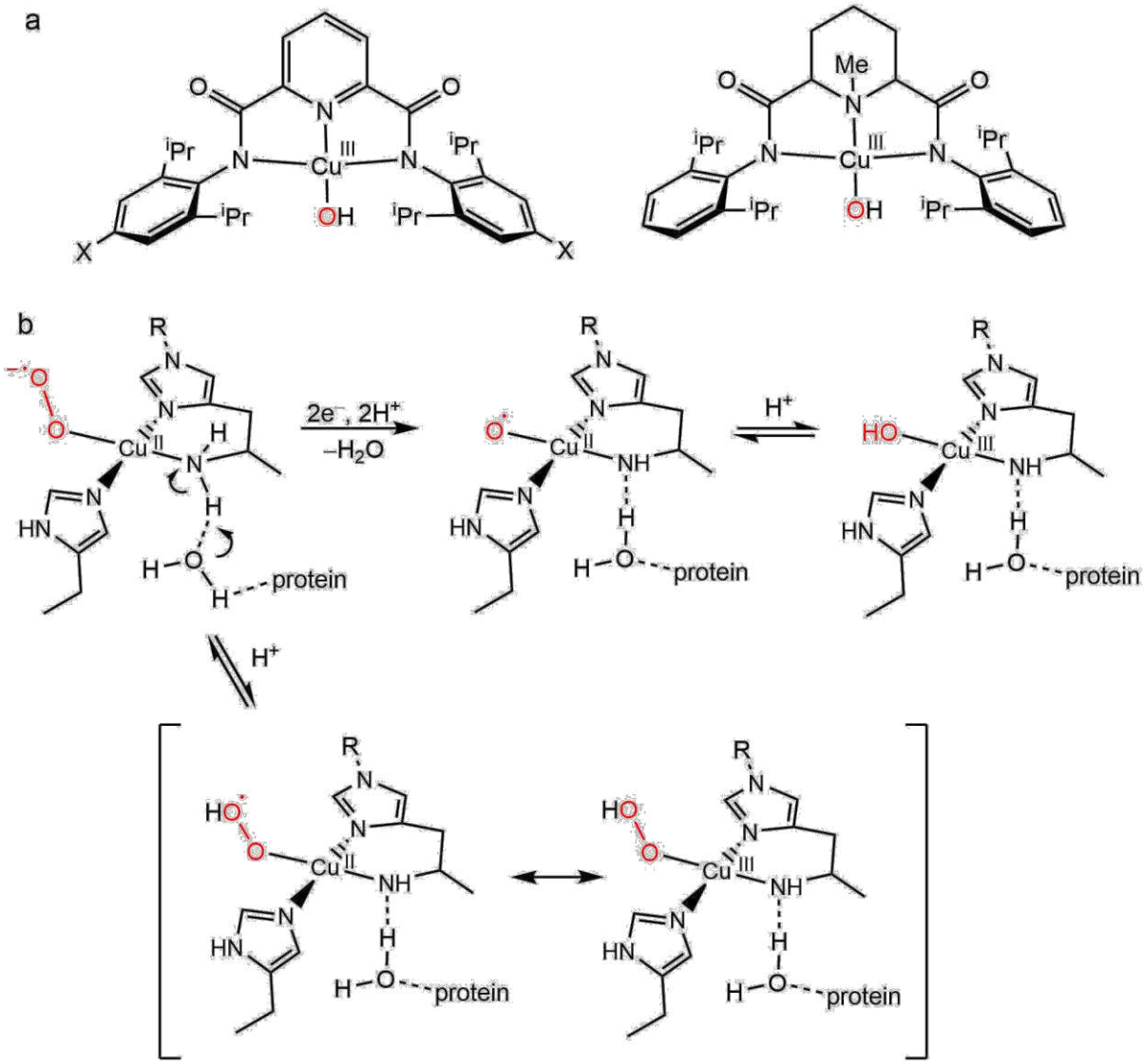

Figure 4. Deprotonation of the amino terminus. a) Complexes with the $[CuOH]^{2+}$ core (X = H or NO_2), b)Proposed intermediates in LPMO highlighting the possible stabilising role of the deprotonated histidine brace (R= H or CH_3). Only ligands in the equatorial plane are shown.

Conclusions

The work on LPMOs, pMMOs, and small molecule copper-oxygen complexes now brings into a new light the oxidative power of mononuclear copper species, especially those in which the histidine brace is part of the coordination sphere. The histidine brace appears to be a key common structural element that may facilitate the formation of and stabilise high-valent intermediates, potentially *via* deprotonation of the amino unit. The message from these studies is that monomeric copper sites, especially those which have a histidine brace coordination geometry, are powerful oxidation catalysts, ones that Nature has already recruited for use in the oxidation of recalcitrant biomass and ones that coordination chemists should now see as an attractive and fruitful target for their own studies.

Acknowledgements

WBT thanks the US National Institute of Health (grant R37GM47365) for financial support. LC, GJD and PHW gratefully acknowledge the support of the UK's Biotechnology and Biological Sciences Research Council (BBSRC) through grants BB/L001926/1 and BB/L021633/1. GJD is a Royal Society Ken Murray Research Professor.

Author contributions

WBT and PHW wrote the manuscript, GJD analysed LPMO and MMO protein structures and contributed to the writing of the manuscript. LC prepared metrical data for structure analysis and contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

References

1. Quinlan, R.J.*, et al.* Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. *Proc. Natl. Acad. Sci. USA* **108**, 15079-15084 (2011).

2. Phillips, C.M., Beeson, W.T., Cate, J.H., Marletta, M.A. Cellobiose Dehydrogenase and a Copper-Dependent Polysaccharide Monooxygenase Potentiate Cellulose Degradation by Neurospora crassa. *ACS Chem. Biol.* **6**, 1399-1406 (2011).

3. Zhang, L.*, et al.* Intermolecular Transfer of Copper Ions from the CopC Protein of Pseudomonas syringae. Crystal Structures of Fully Loaded $Cu^{I}Cu^{II}$ Forms. *J. Am. Chem. Soc.* **128**, 5834-5850 (2006).

4. Vaaje-Kolstad, G.*, et al.* An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. *Science* **330**, 219-222 (2010).

5. Hemsworth, G.R., Davies, G.J., Walton, P.H. Recent insights into copper-containing lytic polysaccharide mono-oxygenases. *Curr. Opin. Struct. Biol.* **23**, 660-668 (2013).

6. Walton, P.H., Davies, G.J. On the catalytic mechanisms of lytic polysaccharide monooxygenases. *Curr. Opin. Chem. Biol.* **31**, 195-207 (2016).

7. Gao, J., Thomas, D.A., Sohn, C.H., Beauchamp, J.L. Biomimetic Reagents for the Selective Free Radical and Acid–Base Chemistry of Glycans: Application to Glycan Structure Determination by Mass Spectrometry. *J. Am. Chem. Soc.* **135**, 10684-10692 (2013).

8. Vu, V.V., Ngo, S.T. Copper active site in polysaccharide monooxygenases. *Coord. Chem. Rev.* **368**, 134-157 (2018).

9. Lieberman, R.L., Rosenzweig, A.C. Crystal structure of a membrane-bound metalloenzyme that catalyses the biological oxidation of methane. *Nature* **434**, 177-182 (2005).

10. Luo, Y.-R. *Comprehensive handbook of chemical bond energies*. CRC press (2007).

11. Solomon, E.I.*, et al.* Copper Active Sites in Biology. *Chem. Rev.* **114**, 3659-3853 (2014).

12. Vaaje-Kolstad, G.*, et al.* The Non-catalytic Chitin-binding Protein CBP21 from Serratia marcescens Is Essential for Chitin Degradation. *J. Biol. Chem.* **280**, 28492-28497 (2005).

13. Vaaje-Kolstad, G.*, et al.* Crystal Structure and Binding Properties of the Serratia marcescens Chitin-binding Protein CBP21. *J. Biol. Chem.* **280**, 11313-11319 (2005).

14. Harris, P.V.*, et al.* Stimulation of Lignocellulosic Biomass Hydrolysis by Proteins of Glycoside Hydrolase Family 61: Structure and Function of a Large, Enigmatic Family. *Biochemistry* **49**, 3305-3316 (2010).

15. Karkehabadi, S.*, et al.* The first structure of a glycoside hydrolase family 61 member, Cel61B from Hypocrea jecorina, at 1.6 Å resolution. *J. Mol. Biol.* **383**, 144-154 (2008).

16. Hemsworth, G.R., Johnston, E.M., Davies, G.J., Walton, P.H. Lytic Polysaccharide Monooxygenases in Biomass Conversion. *Trends Biotechnol.* **33**, 747-761 (2015).

17. Levasseur, A.*, et al.* Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. *Biotechnology for Biofuels* **6**, 41 (2013).

18. Hemsworth, G.R., Henrissat, B., Davies, G.J., Walton, P.H. Discovery of a new family of lytic polysaccharide mono-oxygenases. *Nat. Chem. Biol.* **10**, 122-126 (2014).

19. Vu, V.V.*, et al.* A family of starch-active polysaccharide monooxygenases. *Proc. Natl. Acad. Sci. USA* **111**, 13822-13827 (2014).

20. Lo Leggio, L.*, et al.* Structure and boosting activity of a starch-degrading lytic polysaccharide monooxygenase. *Nat. Commun.* **6**, 5961 (2015).

21. Couturier, M.*, et al.* Lytic xylan oxidases from wood-decay fungi unlock biomass degradation. *Nat. Chem. Biol.* **14**, 306-310 (2018).

22. Sabbadin, F.*, et al.* An ancient family of lytic polysaccharide monooxygenases with roles in arthropod development and biomass digestion. *Nat. Commun.* **9**, 756 (2018).

23. Meier, K.K.*, et al.* Oxygen Activation by Cu LPMOs in Recalcitrant Carbohydrate Polysaccharide Conversion to Monomer Sugars. *Chem. Rev.* **118**, 2593-2635 (2017).

24. Vaaje-Kolstad, G.*, et al.* Structural diversity of lytic polysaccharide monooxygenases. *Curr. Opin. Struct. Biol.* **44**, 67-76 (2017).

25. Span, E.A.*, et al.* The Role of the Secondary Coordination Sphere in a Fungal Polysaccharide Monooxygenase. *ACS Chem. Biol.* **12**, 1095-1103 (2017).

26. Mann, S.I., Heinisch, T., Ward, T.R., Borovik, A.S. Peroxide Activation Regulated by Hydrogen Bonds within Artificial Cu Proteins. *J. Am. Chem. Soc.* **139**, 17289-17292 (2017).

27. Glyn R. Hemsworth, E.J.T., Robbert Q. Kim, Rebecca C. Gregory, Sally J. Lewis, Johan P. Turkenburg, Alison J. Parkin, Gideon J. Davies and Paul H. Walton. The copper active site of CBM33 polysaccharide oxygenases. *J. Am. Chem. Soc.* **135**, 6069-6077 (2013).

28. Forsberg, Z.*, et al.* Structural determinants of bacterial lytic polysaccharide monooxygenase functionality. *J. Biol. Chem.* **293**, 1397-1412 (2018).

29. Frandsen, K.E.H.*, et al.* The molecular basis of polysaccharide cleavage by lytic polysaccharide monooxygenases. *Nat. Chem. Biol.* **12**, 298-303 (2016).

30. Evans, J.P., Ahn, K., Klinman, J.P. Evidence That Dioxygen and Substrate Activation Are Tightly Coupled in Dopamine β-Monooxygenase: Implications for the Reactive Oxygen Species. *J. Biol. Chem.* **278**, 49691-49698 (2003).

31. Sevrioukova, I.F., Poulos, T.L. Understanding the mechanism of cytochrome P450 3A4: recent advances and remaining problems. *Dalton Transactions* **42**, 3116-3126 (2013).

32. Gudmundsson, M.*, et al.* Structural and Electronic Snapshots during the Transition from a Cu(II) to Cu(I) Metal Center of a Lytic Polysaccharide Monooxygenase by X-ray Photoreduction. *J. Biol. Chem.* **289**, 18782-18792 (2014).

33. Hedegard, E.D., Ryde, U. Molecular mechanism of lytic polysaccharide monooxygenases. *Chem. Sci.* **9**, 3866-3880 (2018).

34. Kjaergaard, C.H.*, et al.* Spectroscopic and computational insight into the activation of O_2 by the mononuclear Cu center in polysaccharide monooxygenases. *Proc. Natl. Acad. Sci. USA* **111**, 8797-8802 (2014).

35. Li, X.*, et al.* Structural Basis for Substrate Targeting and Catalysis by Fungal Polysaccharide Monooxygenases. *Structure* **20**, 1051-1061 (2012).

36. McDonald, M.R., Fredericks, F.C., Margerum, D.W. Characterization of Copper(III)−Tetrapeptide Complexes with Histidine as the Third Residue. *Inorg. Chem.* **36**, 3119-3124 (1997).

37. Bacik, J.-P.*, et al.* Neutron and Atomic Resolution X-ray Structures of a Lytic Polysaccharide Monooxygenase Reveal Copper-Mediated Dioxygen Binding and Evidence for N-Terminal Deprotonation. *Biochemistry* **56**, 2529-2532 (2017).

38. Balasubramanian, R.*, et al.* Oxidation of methane by a biological dicopper centre. *Nature* **465**, 115-119 (2010).

39. Lee, J.Y., Karlin, K.D. Elaboration of copper-oxygen mediated C-H activation chemistry in consideration of future fuel and feedstock generation. *Curr. Opin. Chem. Biol.* **25**, 184-193 (2015).

40. Wang, V.C.C.*, et al.* Alkane Oxidation: Methane Monooxygenases, Related Enzymes, and Their Biomimetics. *Chem. Rev.* **117**, 8574-8621 (2017).

41. Sommerhalter, M., Lieberman, R.L., Rosenzweig, A.C. X-ray Crystallography and Biological Metal Centers: Is Seeing Believing? *Inorg. Chem.* **44**, 770-778 (2005).

42. Lieberman, R.L.*, et al.* Characterization of the Particulate Methane Monooxygenase Metal Centers in Multiple Redox States by X-ray Absorption Spectroscopy. *Inorg. Chem.* **45**, 8372-8381 (2006).

43. Culpepper, M.A.*, et al.* Identification of the Valence and Coordination Environment of the Particulate Methane Monooxygenase Copper Centers by Advanced EPR Characterization. *J. Am. Chem. Soc.* **136**, 11767-11775 (2014).

44. Culpepper, M.A., Cutsail, G.E., Hoffman, B.M., Rosenzweig, A.C. Evidence for oxygen binding at the active site of particulate methane monooxygenase. *J. Am. Chem. Soc.* **134**, 7640-7643 (2012).

45. Itoyama, S.*, et al.* Possible Peroxo State of the Dicopper Site of Particulate Methane Monooxygenase from Combined Quantum Mechanics and Molecular Mechanics Calculations. *Inorg. Chem.* **55**, 2771-2775 (2016).

46. Yumura, T.*, et al.* Roles of Water Molecules in Modulating the Reactivity of Dioxygen-Bound Cu-ZSM-5 toward Methane: A Theoretical Prediction. *ACS Catalysis* **6**, 2487-2495 (2016).

47. Yoshizawa, K., Shiota, Y. Conversion of Methane to Methanol at the Mononuclear and Dinuclear Copper Sites of Particulate Methane Monooxygenase (pMMO): A DFT and QM/MM Study. *J. Am. Chem. Soc.* **128**, 9873-9881 (2006).

48. Citek, C., Herres-Pawlis, S., Stack, T.D.P. Low Temperature Syntheses and Reactivity of Cu2O2 Active-Site Models. *Acc. Chem. Res.* **48**, 2424-2433 (2015).

49. Elwell, C.E.*, et al.* Copper–Oxygen Complexes Revisited: Structures, Spectroscopy, and Reactivity. *Chem. Rev.* **117**, 2059-2107 (2017).

50. Smith, S.M.*, et al.* Crystal Structure and Characterization of Particulate Methane Monooxygenase from Methylocystis species Strain M. *Biochemistry* **50**, 10231-10240 (2011).

51. Cao, L., Caldararu, O., Rosenzweig, A.C., Ryde, U. Quantum Refinement Does Not Support Dinuclear Copper Sites in Crystal Structures of Particulate Methane Monooxygenase. *Angew. Chem. Int. Ed.* **57**, 162-166 (2018).

52. Ro, S.Y.*, et al.* From micelles to bicelles: Effect of the membrane on particulate methane monooxygenase activity. *J. Biol. Chem.*, epub ahead of print: 10.1074/jbc.RA1118.003348 (2018).

53. Allen, S.E., Walvoord, R.R., Padilla-Salinas, R., Kozlowski, M.C. Aerobic Copper-Catalyzed Organic Reactions. *Chem. Rev.* **113**, 6234-6458 (2013).

54. Bissaro, B.*, et al.* Oxidative cleavage of polysaccharides by monocopper enzymes depends on H_2O_2. *Nat. Chem. Biol.* **13**, 1123-1128 (2017).

55. Hangasky, J.A., Iavarone, A.T., Marletta, M.A. Reactivity of O_2 versus H_2O_2 with polysaccharide monooxygenases. *Proc. Natl. Acad. Sci. USA*, epub ahead of print: 10.1073/pnas.1801153115 (2018).

56. Kim, S.*, et al.* Amine Oxidative N-Dealkylation via Cupric Hydroperoxide Cu-OOH Homolytic Cleavage Followed by Site-Specific Fenton Chemistry. *J. Am. Chem. Soc.* **137**, 2867-2874 (2015).

57. Mirica, L.M., Ottenwaelder, X., Stack, T.D.P. Structure and Spectroscopy of Copper−Dioxygen Complexes. *Chem. Rev.* **104**, 1013-1046 (2004).

58. Lewis, E.A., Tolman, W.B. Reactivity of Dioxygen−Copper Systems. *Chem. Rev.* **104**, 1047-1076 (2004).

59. Liu, J.J., Diaz, D.E., Quist, D.A., Karlin, K.D. Copper(I)-Dioxygen Adducts and Copper Enzyme Mechanisms. *Isr. J. Chem.* **56**, 9-10 (2016).

60. Quist, D.A., Diaz, D.E., Liu, J.J., Karlin, K.D. Activation of dioxygen by copper metalloproteins and insights from model complexes. *J. Biol. Inorg. Chem.* **22**, 253-288 (2017).

61. Cramer, C.J., Tolman, W.B. Mononuclear Cu–O_2 Complexes: Geometries, Spectroscopic Properties, Electronic Structures, and Reactivity. *Acc. Chem. Res.* **40**, 601-608 (2007).

62. Itoh, S. Developing Mononuclear Copper–Active-Oxygen Complexes Relevant to Reactive Intermediates of Biological Oxidation Reactions. *Acc. Chem. Res.* **48**, 2066-2074 (2015).

63. Gagnon, N., Tolman, W.B. $[CuO]^{+}$ and $[CuOH]^{2+}$ Complexes: Intermediates in Oxidation Catalysis? *Acc. Chem. Res.* **48**, 2126-2131 (2015).

64. Iovan, D.A.*, et al.* Reactivity of a stable copper-dioxygen complex. *Chem. Commun.* **53**, 10306-10309 (2017).

65. Peterson, R.L.*, et al.* Cupric Superoxo-Mediated Intermolecular C-H Activation Chemistry. *J. Am. Chem. Soc.* **133**, 1702-1705 (2011).

66. Tomson, N.C.*, et al.* Re-evaluating the Cu K pre-edge XAS transition in complexes with covalent metal-ligand interactions. *Chem. Sci.* **6**, 2474-2487 (2015).

67. Hedegård, E.D., Ryde, U. Targeting the reactive intermediate in polysaccharide monooxygenases. *J. Biol. Inorg. Chem.* **22**, 1029-1037 (2017).

68. Wang, B.*, et al.* QM/MM Studies into the H_2O_2-Dependent Activity of Lytic Polysaccharide Monooxygenases: Evidence for the Formation of a Caged Hydroxyl Radical Intermediate. *ACS Catalysis* **8**, 1346-1351 (2018).

69. Neisen, B.D.*, et al.* Formally Copper(III)–Alkylperoxo Complexes as Models of Possible Intermediates in Monooxygenase Enzymes. *J. Am. Chem. Soc.* **139**, 10220-10223 (2017).

70. Yamaguchi, S., Masuda, H. Basic approach to development of environment-friendly oxidation catalyst materials. Mononuclear hydroperoxo copper(II) complexes. *Science and Technology of Advanced Materials* **6**, 34-47 (2005).

71. Kitajima, N.*, et al.* Synthesis, molecular structure, and reactivity of (alkylperoxo)copper(II) complex. *J. Am. Chem. Soc.* **115**, 7872-7873 (1993).

72. Peterson, R.L.*, et al.* Stepwise Protonation and Electron-Transfer Reduction of a Primary Copper–Dioxygen Adduct. *J. Am. Chem. Soc.* **135**, 16454-16467 (2013).

73. Trammell, R.*, et al.* Decoding the Mechanism of Intramolecular Cu-Directed Hydroxylation of sp3 C–H Bonds. *J. Org. Chem.* **82**, 7887-7904 (2017).

74. Maiti, D.*, et al.* Reactions of a copper(II) superoxo complex lead to C-H and O-H substrate oxygenation: modeling copper-monooxygenase C-H hydroxylation. *Angew. Chem. Int. Ed.* **47**, 82-85 (2008).

75. Tano, T.*, et al.* Reactivity of copper(II)-alkylperoxo complexes. *Dalton transactions (Cambridge, England : 2003)* **40**, 10326-10336 (2011).

76. Bertini, L.*, et al.* Catalytic Mechanism of Fungal Lytic Polysaccharide Monooxygenases Investigated by First-Principles Calculations. *Inorg. Chem.* **57**, 86-97 (2018).

77. Osborne, R.L., Klinman, J.P. in: *Copper-Oxygen Chemistry* (eds Karlin, K.D., Itoh, S.), Ch. 1, pp 1-22, (John Wiley & Sons, 2011).

78. Dietl, N.*, et al.* Diatomic $[CuO]^+$ and its role in the spin-selective hydrogen- and oxygen-atom transfers in the thermal activation of methane. *Angew. Chem. Int. Ed.* **50**, 4966-4969 (2011).

79. Schröder, D., Holthausen, M.C., Schwarz, H. Radical-Like Activation of Alkanes by the Ligated Copper Oxide Cation (Phenanthroline)CuO^+. *J. Phys. Chem. B* **108**, 14407-14416 (2004).

80. Yoshizawa, K., Kihara, N., Kamachi, T., Shiota, Y. Catalytic Mechanism of Dopamine β-Monooxygenase Mediated by Cu(III)−Oxo. *Inorg. Chem.* **45**, 3034-3041 (2006).

81. Dietl, N., Schlangen, M., Schwarz, H. Thermal Hydrogen-Atom Transfer from Methane: The Role of Radicals and Spin States in Oxo-Cluster Chemistry. *Angew. Chem. Int. Ed.* **51**, 5544-5555 (2012).

82. Donoghue, P.J.*, et al.* Rapid C–H Bond Activation by a Monocopper(III)–Hydroxide Complex. *J. Am. Chem. Soc.* **133**, 17602-17605 (2011).

83. Dhar, D., Tolman, W.B. Hydrogen Atom Abstraction from Hydrocarbons by a Copper(III)-Hydroxide Complex. *J. Am. Chem. Soc.* **137**, 1322-1329 (2015).

84. Dhar, D.*, et al.* Perturbing the Copper(III)–Hydroxide Unit through Ligand Structural Variation. *J. Am. Chem. Soc.* **138**, 356-368 (2016).

85. Dhar, D.*, et al.* Reactivity of the Copper(III)-Hydroxide Unit with Phenols. *Chem. Sci.* **8**, 1075-1085 (2017).

86. Spaeth, A.D.*, et al.* Determination of the Cu(III)–OH Bond Distance by Resonance Raman Spectroscopy Using a Normalized Version of Badger's Rule. *J. Am. Chem. Soc.* **139**, 4477-4485 (2017).

87. Donoghue, P.J.*, et al.* An Anionic, Tetragonal Copper(II) Superoxide Complex. *J. Am. Chem. Soc.* **132**, 15869-15871 (2010).

88. Pirovano, P.*, et al.* Nucleophilic Reactivity of a Copper(II)–Superoxide Complex. *Angew. Chem. Int. Ed.* **126**, 6056-6060 (2014).